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TESTS OF THE CARRIER MODEL FOR ION TRANSPORT BY NONACTIN AND TRINACTIN

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SUMMARY

The fluxes of K^+ and NH_4^+ carried by nonactin and trinactin across thin lipid membranes have been measured as functions of ion activity, electric potential and time. In agreement with the predictions of a version of the carrier model in common use, the shape of the initial current–voltage relation is independent of the activity of the electrolyte, a_i, while the ratio of the initial conductance, G_0, to the steady-state conductance, G_∞, increases according to $G_0/G_\infty = \text{const}_1 + \text{const}_2 \times a_i$. For trinactin the data presented allow the estimation of the rate constants of the carrier process (in the limit of zero potential) in a manner which does not assume any particular variation with potential for the constants. Using empirically determined functions of potential, a complete set of values is also available for nonactin. The curve fitting which is necessary is described in the following paper. The data presently available for valinomycin are sufficient neither to test the model nor to determine a complete set of constants.

INTRODUCTION

There is a large body of evidence (reviewed in refs 1–4) which establishes that the macrotetralide actins and valinomycin transport an ion across the hydrocarbon core of thin lipid membranes as part of a mobile one ion-one carrier complex. Furthermore, one carrier molecule transports many ions while it remains associated with the membrane [4–6] and variations in the concentration of carrier associated with the membrane occur slowly [3, 7, 8] and thus can be ignored in the rapid comparisons of fluxes at different potentials. The simplest model which predicts these observations is one in which (i) the complexes and carriers are adsorbed to the surface of the membrane, (ii) each flux across the membrane increases linearly with the concentrations of the moving species, (iii) association of an ion with a carrier is first order in the ion activity and first order in the carrier concentration, (iv) dissociation of a complex is first order, and (v) the fluxes of carriers and complexes into and out of

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* Most of the work reported in this paper was carried out while the author was a Beit Memorial Research Fellow.

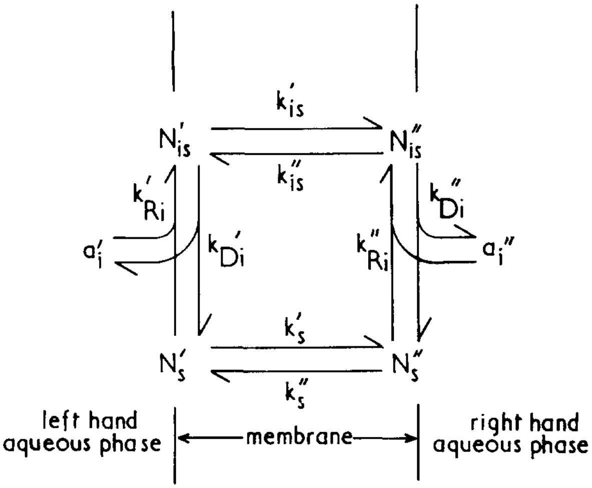

Fig. 1. A simple version of the carrier model. The surface concentration of carrier (subscripts) and complex (is) on the left hand (single prime) and right hand (double prime) sides of the membrane are denoted by N's (moles/cm^2). The molal activity of the electrolyte (with units hereafter abbreviated) [act] is a_i. The rate constants are defined in the text.

the membrane are negligible compared to the fluxes across the membrane. This model (Fig. 1) has been discussed frequently in the literature [1, 2, 9–15]. The notation used here (see Fig. 1) is the same as that used in ref. 12 and is a modification of that introduced by Läuger and Stark [10]. My purpose in this paper is to test this model and to determine the values of the various rate constants.

Extensive data are required if the model is to be both specified and tested since initially there are five unknowns; the concentration of the unbound carrier in the membrane, $2N_s$, and the rate constants for the four steps in the process: the association reaction at a given ion activity, $k_{Ri}a_i$, the transfer of complexes across the membrane, k_{is}, the dissociation of the complexes, k_{Di}, and the transfer of free carrier across the membrane, k_s. A priori all the rate constants can vary with the applied potential. Thus there is considerable flexibility available to allow adjustment of the theoretical predictions to fit the data. Nevertheless there are three predictions of the model which can be tested.

With the same electrolyte activity in the two aqueous phases and zero applied potential, the carrier concentration on the two sides of the membrane will be the same, $N'_s = N''_s = N_s$, and similarly for the concentration of complexes, $N'_{is} = N''_{is} = N_{is} = (k_{Ri}\, a_i/k_{Di})\, N_s$. It is an essential and characteristic feature of the model that after the sudden application of a potential there will be a net flux of complexes across the membrane which will relax from an initial value,

$$J_{is} = N_{is}(k'_{is} - k''_{is}) \tag{1}$$

to a final steady-state value as the carrier is redistributed across the membrane and as the balance is altered between the complexed and free forms. These changes may occur both as a result of a direct effect of the applied potential on the equilibrium constant for the formation of complexes and because the flux across the membrane may remove complexes more rapidly than they can be formed and may sweep all of the carrier to one side. Whatever relaxations may occur, since k'_{is} and k''_{is} are assumed not to depend on electrolyte activity, it follows immediately from Eqn 1 that the ratio of the initial current at any given potential to that at some reference potential should be independent of electrolyte activity. This independence is the first testable prediction of the theory.

The steady-state solution of the equations which describe the model has been presented elsewhere [12]. The conductance at zero applied potential is given by the expression [10, 12, 14]

$$G_{\infty}(0) = \left(\frac{z_{is}^2 F^2}{RT}\right)\left(\frac{k_{Ri}a_i}{k_{Di}}\right) N_s k_{is} \Big/ \left[1+\frac{2k_{is}}{k_{Di}}+\frac{k_{is}k_{Ri}}{k_s k_{Di}}a_i\right] \tag{2}$$

where z_{is} is the charge on the complex, F is the Faraday, R the gas constant, and T the absolute temperature. From Eqns 1 and 2 the ratio of the initial conductance to the steady state conductance is simply

$$\alpha+1 = G_0/G_{\infty} = 1+2k_{is}/k_{Di}+(k_{is}k_{Ri}/k_s k_{Di})a_i \tag{3}$$

This relation is the second testable prediction.

The time course of the change in conductance from the initial to the steady-state value depends upon all of the rate constants. However, from the measured time course (in the limit of zero applied potential) at one electrolyte activity, the values of k_{is}/k_{Di} and $k_{is}\ k_{Ri}/k_s\ k_{Di}$ determined by fitting Eqn 3 to the data, the value of N_s from adsorption measurements, and the steady-state conductance, it is possible to calculate the time course at all other activities. The results of these calculations are the third prediction.

Fortunately, using nonactin and trinactin as carriers, KCl and NH_4Cl as electrolytes and glyceryl monooleate+n-hexadecane membranes, it is possible to obtain sufficiently extensive data to check these predictions and to determine approximate values for the rate constants.

THEORY

Mathematical expressions for the current and the concentrations, N'_s, N''_s, N'_{is}, and N''_{is}, at any instant after a sudden change in potential could be derived using established techniques starting from Eqns A-1 to A-5 in Appendix I. However, the results would be so complicated that they would convey little more information than is available from a careful inspection of the differential equations and the use of certain simplifying assumptions. By far the most important of these is the special case considered by Stark et al. [11]. If the rates of association and dissociation are the same on both sides of the membrane and independent of the applied potential,

$$k'_{Ri}a'_i = k''_{Ri}a''_i = k_{Ri}a_i \tag{4}$$

and

$$k'_{Di} = k''_{Di} = k_{Di}, \tag{5}$$

then the proportion of the carrier present as complex is independent of the applied potential and of the distribution of the carrier across the membrane, i.e. (see Appendix I)

$$N'_s + N''_s = 2N_s \tag{6}$$

and

$$N'_{is} + N''_{is} = 2(k_{Ri}\,a_i/k_{Di})N_s\,. \tag{7}$$

Thus, specifically as a result of these additional assumptions (Eqns 4 and 5) there will be only two unknowns, which may be chosen as N'_{is} and N'_s. The solution to the equations governing these variables is outlined in Appendix I with the additional assumption that $k'_s = k''_s$. The result is of course the same as that given by Stark et al. [11] except that the potential dependence of $k'_{is} + k''_{is}$ is left unspecified.

Under the above assumptions the theory predicts two relaxations (i.e. two declining exponential terms in the expression for the current) even for low applied potentials. The experimental data, however, display only one. The principal difficulty in applying the theory to the data is thus the identification of the observed relaxation either with one of the predicted relaxations or with some combination which cannot be resolved into its components. In this respect it is useful to have still more compact expressions for the amplitudes, α_1 and α_2, and time constants, τ_1 and τ_2, even though these expressions are of still less general applicability. Such expressions are available for

$$\text{I:}\ \ k_{Ri}\,a_i + 2k_s \gg k'_{is} + k''_{is} + k_{Di} \tag{8}$$

and

$$\text{II:}\ \ k'_{is} + k''_{is} + k_{Di} \gg 2k_s + k_{Ri}\,a_i\,. \tag{9}$$

The first inequality may be obeyed either because the flux of free carrier is large or, because the rate of association is fast. In either of these instances, the slower relaxation dominates,

$$\alpha_1 = \alpha = [(k'_{is} + k''_{is})/k_{Di}][1 + k_{Ri}\,a_i/2k_s] \tag{10}$$

and

$$\frac{1}{\tau_1} = (k'_{is} + k''_{is})(\alpha + 1)/\alpha \tag{11}$$

When the opposite inequality (Eqn 9) holds, for instance when dissociation of the complex is rapid, there may be one or two relaxations visible:

$$\alpha_1 = [(k'_{is} + k''_{is})/(k'_{is} + k''_{is} + k_{Di})][k_{Ri}\,a_i/2k_s], \tag{12}$$

$$\frac{1}{\tau_1} = 2k_s(1 + \alpha_1), \tag{13}$$

$$\alpha_2 = [(k'_{is} + k''_{is})/k_{Di}][1 + k_{Ri}\,a_i/2k_s] - \alpha_1\,, \tag{14}$$

$$\frac{1}{\tau_2} = k'_{is} + k''_{is} + k_{Di}\,. \tag{15}$$

If $k'_{is} + k''_{is} \gg k_{Di}$ then the second (faster) relaxation is much larger than the first and it has the same appearance as in Case I, while if $k_{Di} \gg k'_{is}+k''_{is}$ no relaxation is visible for low electrolyte concentrations (i.e. $\alpha \ll 1$) and as the electrolyte concentration increases the slower relaxation amplitude becomes larger and the associated time constant more strongly dependent on k_{is} and thus on the applied potential. For $k'_{is}+k''_{is} \simeq k_{Di}$ there are two relaxations which for $k_{Ri}a_i/2k_s \simeq 1$ are of comparable amplitude with the slower relaxation only weakly dependent on k_{is}.

The preceding expressions are valid only for sufficiently small applied potentials that the rates of dissociation, association, and transfer of free carrier may be presumed to be constants. Approximations valid at all potentials are set out in the appendix for the limits of high and low electrolyte activity.

METHODS

The materials and procedures used were the same as those described in an earlier paper of this series [15] with only minor alterations to allow the detection of rapid changes in current. Membranes were formed from 7 mM glyceryl monooleate in *n*-hexadecane and were spread across a 1- or 1.5-mm hole in a Teflon cup. Steps in the applied potential were produced by an electronic switch with the output taken via either a resistance divider for small currents or the divider and a unity gain follower for larger currents ($\geqslant$ or $\approx 10^{-7}$ A) in order to insure a rise time of approx. 200 ns. The currents were measured using an Analogue Devices 48K amplifier in a virtual earth configuration [16], which allows both a high speed of response and the maintenance of constant potential across the electrodes. In order to reduce solution resistance the ionic strength of the aqueous phases was always kept above 1 M by the addition of LiCl. As expected, since glyceryl monooleate is neutral, the ionic strength has only a minor effect on the conductances which can be accounted for by the change in the activity coefficients. The aqueous phases were not buffered; the temperature was 22 °C. Nonactin (gift of Dr B. Stearns, Squibb) and trinactin (gift of Dr H. Bickel, Ciba-Geigy) were each dissolved in methanol at 10^{-3} M. The appropriate amount of stock solution was added to a small vial, the methanol was removed using a rotary evaporator, and the lipid solution was added to give an actin concentration between 10^{-5} and 10^{-3} M. Using 6 M NH_4Cl the potential across the Ag–AgCl electrodes drifts, but the zero current potential is the same whether or not a membrane separates the two compartments. The relaxations are reported as a function of the difference between the applied and zero current potentials and are symmetrical about this value. Because of the capacitative transient it has not been possible to obtain the amplitudes of any relaxations with time constants less than about 5 μs which limits the range of potentials that may be investigated.

RESULTS

In all instances the observed current after the capacity transient could be fitted with a constant plus a single exponential (Figs 2 and 3). The observed amplitudes of the exponential term and the time constants are listed in Tables I and II. At simultaneously high carrier and electrolyte concentrations (e. g. 1 M KCl or NH_4Cl and 10^{-3} M trinactin) the relaxations were considerably smaller and slower than those reported in the tables. The explanation may be partially that at high currents

TABLE I

TIME CONSTANT, τ, IN μs AND RELATIVE AMPLITUDE, α, FOR THE OBSERVED RELAXATION WITH NONACTIN

Applied potential (mV)	Electrolyte used and concns of nonactin (M)						
	0.1 M KCl 10^{-4}, 10^{-3}	1 M KCl 10^{-4}		4 molal KCl 10^{-5}, 10^{-4}		0.01 M NH_4Cl 10^{-3}	
		τ	α	τ	α	τ	α
25	Any relaxation is such that $\frac{I(8\,\mu s) - I_\infty}{I_\infty} < 0.03$	10–12	0.1–0.3	13–16	0.5–0.65	—	0.2–0.55
50		6–10	0.15–0.4	9–15	0.5–0.65	25–30	0.2–0.5
100		6–10	0.3–0.65	8–10	0.9–2	19–20	0.7–1.2
150		6–7.5	0.65–0.85	6–7	1.7–3.5	12	2.8

Applied potential (mV)	Electrolyte used and concns of nonactin (M)							
	0.1 M NH_4Cl 10^{-4}, 10^{-3}		1 M NH_4Cl 10^{-5}, 10^{-4}		2 molal NH_4Cl 10^{-5}		6 molal NH_4Cl 10^{-5}	
	τ	α	τ	α	τ	α	τ	α
25	31–32	0.3–0.7	44–52	0.9–2	49–53	2–2.3	50–56	6–6.9
50	22–32	0.4–0.6	40–45	0.9–2.2	43–47	2.5–2.8	43–49	7–9
100	17–21	0.8–1	22–27	1.6–3.4	26–29	4.9–5	22–30	8–20
150	11–14	1.9–2.4	15–16	4–7	14	11.5	16	15–23

TABLE II

TIME CONSTANT, τ, IN μs AND RELATIVE AMPLITUDE, α, FOR THE OBSERVED RELAXATION WITH TRINACTIN

Applied potential (mV)	Electrolyte used and concns of trinactin (M)									
	0.01 M KCl 10^{-4}, 10^{-3}		0.1 M KCl 10^{-4}		0.5 M KCl 10^{-5}, $4 \cdot 10^{-5}$		1 M KCl 10^{-5}		4 molal KCl 10^{-5}, $4 \cdot 10^{-5}$	
	τ	α	τ	α	τ	α	τ	α	τ	α
25	10	0.7–1.5	11	0.7–1.4	14	1.3–1.8	15–16	2–3	14–17	7–9
50	9	1.1	11	0.9	11–12	1.9–2.2	13–15	2.8–3	12–15	9–11
100	6.7	2.2	7	2.6	6.7–7.5	4–4.4	9	5–6.5	8–8.6	15–18
150	4.6	4	4.5	6.8	5	8.3	5	11	4–6	23–35

Applied potential (mV)	Electrolyte used and concns of trinactin (M)											
	0.01 M NH_4Cl 10^{-4}, 10^{-5}		0.02 M NH_4Cl 10^{-5}, 10^{-4}, 10^{-3}		0.1 M NH_4Cl 10^{-5}, 10^{-4}		1 M NH_4Cl 10^{-5}		2 molal NH_4Cl 10^{-5}		6 molal NH_4Cl 10^{-5}, 10^{-6}	
	τ	α	τ	α	τ	α	τ	α	τ	α	τ	α
25	23–27	2.7–4.6	22–26	2.7–4.6	22–27	3.7–5.0	28–36	9–11	28–34	11–16	28	35–45
50	20–25	4.2–6.1	20–22	3–4.2	21–23	3.7–5.6	24–32	9–11	25–32	12–18	23–24	50–60
100	11–14	8.5–13.2	12.5–13.5	5.5–6.7	14	6–10	15–17	17–20	14–17	22–33	13	85
150	7	21	7	11.5	7–8	9–20	8–9	30–35	7–10	50–60	7	155

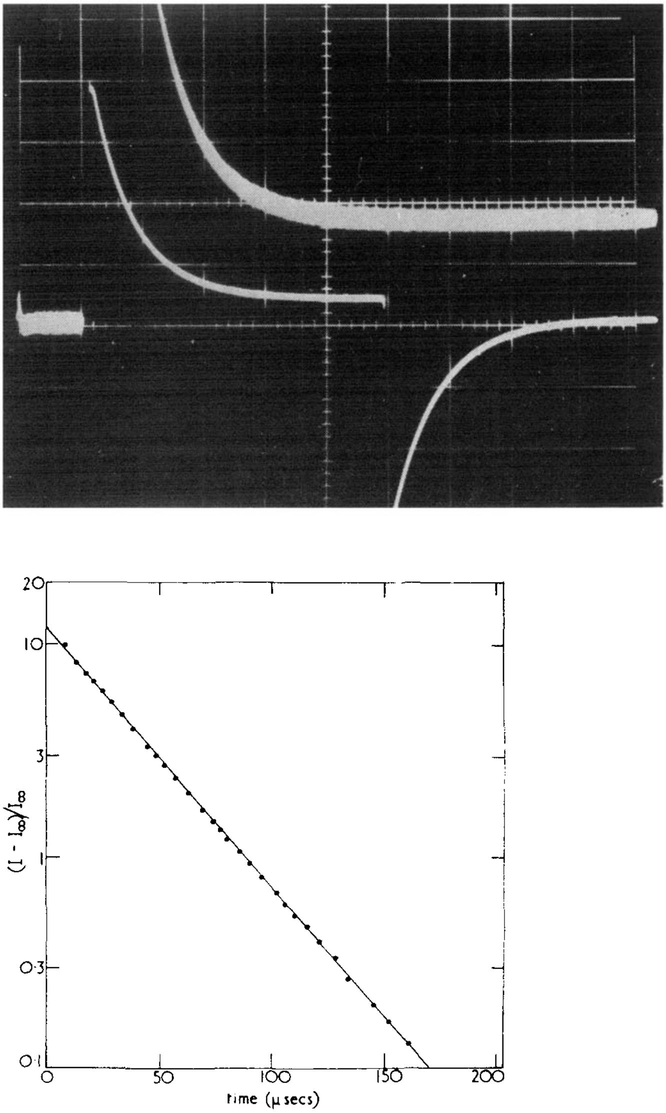

Fig. 2. The current following the sudden application of a 25 mV applied potential for 10^{-5} M trinactin both in the lipid and preloaded into the aqueous phase containing 2 molal NH_4Cl. The membrane area was $5 \cdot 10^{-3}$ cm^2. In the photograph one large horizontal unit equals 50 μs and one large vertical unit equals 10^{-6} A for the lower trace and $2 \cdot 10^{-7}$ A for the upper trace. In the lower trace the potential was returned to zero after 250 μs. The capacity transient is complete within the first 10 μs. The experimental data (dots) are fitted with a single exponential, $\tau = 34$ μs, $\alpha = 12$.

the solution and electrode resistances decrease the potential across the membrane. It is more likely to be primarily that a saturation or "space charge" effect occurs in the membrane itself. The amplitudes of the relaxations at 25 mV are presented graphically in Figs 4 and 5. The apparent initial current–voltage relation may be

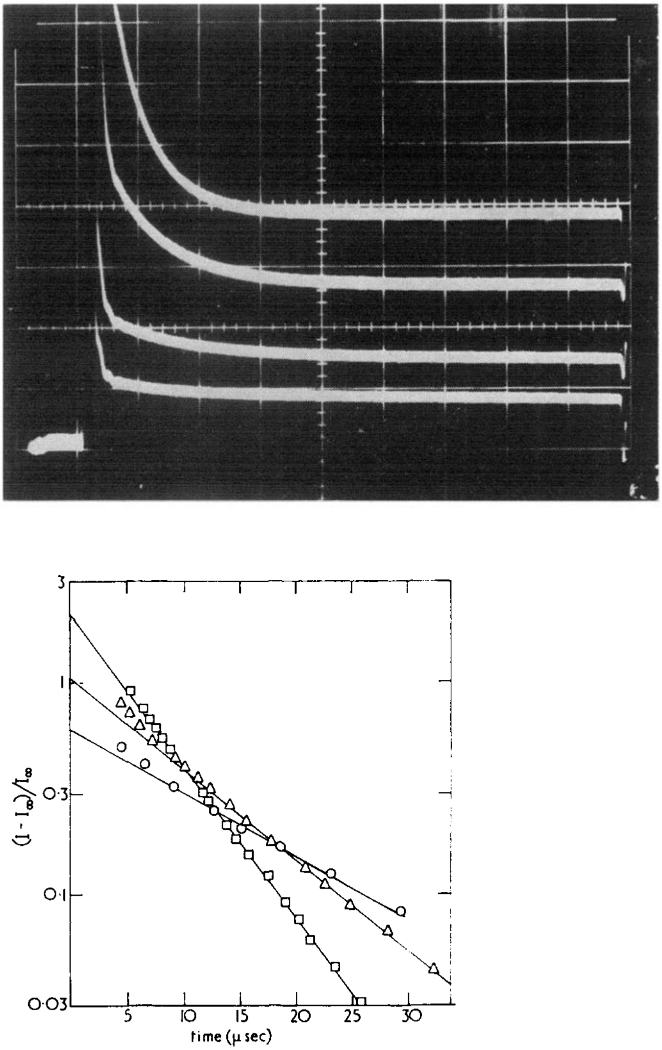

Fig. 3. Relaxations as in Fig. 2 for 4 molal KCl and 10^{-4} M nonactin in the lipid. The four traces, in ascending order, were recorded after potential jumps of 25, 50, 100, and 150 mV. One large division is 10 μs horizontally and $2.5 \cdot 10^{-6}$ A vertically. Further examples of the experimental data have been published as Fig. 5 of [4] and Fig. 26 of [16].

calculated from the relaxation amplitudes if the steady-state current–voltage relation is known since

$$I_0(\Delta V)/I_0(100) = \frac{\alpha(\Delta V)+1}{\alpha(100)+1}\,\frac{G_\infty(\Delta V)}{G_\infty(100)}\,\frac{\Delta V}{100} \tag{16}$$

The values of G_∞ $(\Delta V)/G_\infty$ (100) used in Table III are taken from the experimental data reported in the following paper [17].

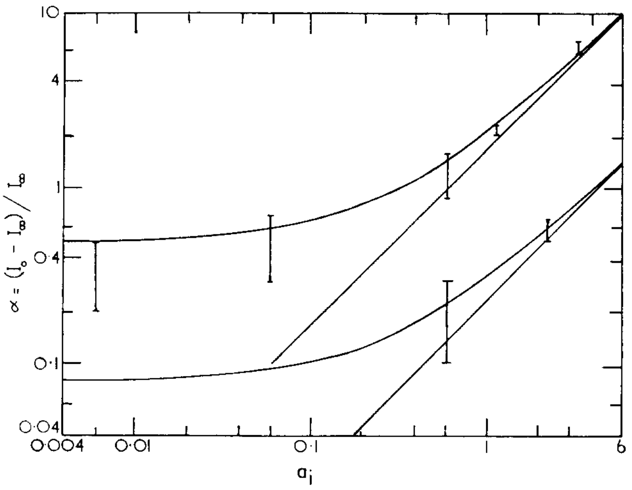

Fig. 4. The relaxation amplitudes for nonactin with various activities of KCl (lower) and NH_4Cl (upper). The theoretical curves have been drawn as described in the text assuming a constant value at low activities and a limiting asymptote of unit slope at high activities, i.e. $\alpha = 2k_{is}/k_{Di} + k_{is}k_{Ri}a_i/k_sk_{Di}$.

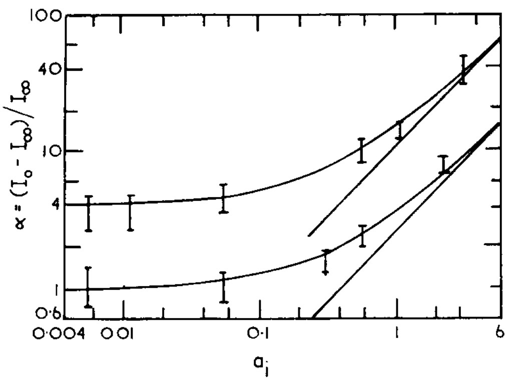

Fig. 5. The relaxation amplitudes for trinactin with various activities of KCl (lower) and NH_4Cl (upper). The theoretical curves were drawn as in Fig. 4.

DISCUSSION

Since only one exponential relaxation can be resolved, it is necessary to determine whether or not there is a large faster relaxation which has escaped detection. That the entire relaxation amplitude has been observed may be seen in two different ways by comparison of the data with the model. Firstly for trinactin and either KCl or NH_4Cl, the amplitude of the observed low potential relaxation approaches a finite limiting value at low electrolyte concentrations (Fig. 5) which according to the theory must be equal to $2k_{is}/k_{Di}$ (cf Eqns 10 and 14). In physical terms, under these conditions there is only one relaxation and it corresponds to the depletion of complexes, at constant free carrier concentration, by the flux across the membrane [15]. Furthermore at high electrolyte concentrations there can be two distinct large relaxations only if $k_s/k_{is}<1$ and the additional relaxation would be seen since it must be slower than the one observed at low activities. Secondly Eqn 1 predicts that $I_0(\Delta V)/I_0(100)$ should be independent of electrolyte activity and, provided the potential dependence of $k'_{is}-k''_{is}$ is the same, that it should be the same for both carriers and both species of cation. This invariance would not apply for a simple carrier unless any undetected relaxations were negligibly small. The current ratios are assembled in Table III from which it may be seen that Eqn 1 is obeyed.

By inspection of Figs 4 and 5 the second prediction of the theory is also seen to be obeyed. From the low activity limits and Eqn 3, it is possible to determine k_{is}/k_{Di} for trinactin with K^+ and NH_4^+ and from the high activity asymptotes $k_{is}\ k_{Ri}/k_S\ k_{Di}$ for nonactin and trinactin. These values are independent of any specific assumptions concerning the potential dependence of the rate constants. More restricted values of k_{is}/k_{Di} for nonactin and trinactin may be derived, as is done in the following paper [17], by fitting the steady-state current–voltage relations assuming only that the same potential dependences for $k'_{Ri}\ k'_{is}/k'_{Di}$ and k'_{Ri} apply for nonactin and trinactin with Na^+, K^+ and NH_4^+. These values are indicated in the figures and are listed in Table V.

The rate constant for the transfer of complex across the membrane, k_{is}, may be calculated numerically (see Appendix I) from the observed values of τ, k_{is}/k_{Di}, $k_{is}\ k_{Ri}/k_{Di}\ k_s$, and k_s/k_{is} but for nonactin–NH_4^+, trinactin–K^+, and trinactin–NH_4^+ it varies only slightly with the value of k_s/k_{is}. The potential dependence of $k'_{is}-k''_{is}$ is shown in Table III; that of $k'_{is}+k''_{is}$ may be estimated from the values of τ at various potentials if either $k'_{is}+k''_{is} \gg k_{Di}$ or $k_{Ri}+2k_s \gg k'_{is}+k''_{is}+k_{Di}$ (see Eqns 11, 15, A-13, A-14 and A-32). In either instance at high potentials α is greater than 1 and thus as indicated in Eqn 11 the assumptions concerning k_{Ri}, k_{Di}, and k_s become less important. The experimental values of $\alpha/(\alpha+1)$ and calculated values of $[k'_{is}(\Delta V)+k''_{is}(\Delta V)]/[k'_{is}(100)+k''_{is}(100)]$ are listed in Table IV. Both the potential dependence from I_0 and that from τ are consistent with the empirical expression

$$k'_{is} = k_{is}\exp\left[-(0.5-\xi)(F\Delta V/RT)-b(F\Delta V/RT)^2\right] \tag{17}$$

where $b = 0.005$ [15] and ξ is approx. 0.1*.

The value of k_s may be related to the concentration of carrier in the membrane by reference to the steady-state conductance data for low electrolyte activities presented elsewhere [15, 17]. For the carrier in the membrane at equilibrium with a concentration, c_l, in the lipid, the same electrolyte activity on both sides, and low potentials, the conductance is given by Eqn 2 with $N_s = \xi_s c_l$ where ξ_s is the lipid phase to membrane adsorption constant. The values of $\xi_s k_{Ri} k_{is}/(k_{Di}+2k_{is})$ are listed in Table V along with the constants determined from the relaxation data. From these it is possible to calculate $\xi_s k_s$ which is seen to vary only slightly for the combinations of nonactin and trinactin with Na^+, K^+ and $NH_4{}^+$.

The ratio of the rate constants for transfer, k_s/k_{is} (and the adsorption constant, ξ_s) cannot be determined in a convincing manner from the flux data.

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* In order to retain the notations used in earlier papers it has been necessary to use both ξ and ξ_s, which should be distinguished, and to have two meanings for the symbol b. The b in Eqn 17 may be recognised in this paper and the following since it occurs solely in conjunction with $(F\Delta V/RT)^2$.

TABLE III

OBSERVED INITIAL CURRENT–VOLTAGE RELATIONS

Under each ion-carrier combination the first column lists $G_\infty(\Delta V)/G_\infty$ (100 mV) taken from the following paper and the second column lists the values of $I_0(\Delta V)/I_0(100)$. While the former ratios vary considerably and systematically, the latter are constant.

ΔV	Nonactin											
	1 M KCl		4 molal KCl		0.1 M NH_4Cl		1 M NH_4Cl		2 molal NH_4Cl		6 molal NH_4Cl	
	G/G_{100}	I/I_{100}	G/G_{100}	I/I_{100}	G/G_{100}	I/I_{100}	G/G_{100}	I/I_{100}	G/G_{100}	I/I_{100}	G/G_{100}	I/I_{100}
25	0.85	0.17	1.04	0.2	1	0.17	1.24	0.21	1.38	0.18	1.39	0.17
50	0.9	0.4	1.04	0.44	1	0.39	1.18	0.42	1.26	0.39	1.26	0.38
100	1	1	1	1	1	1	1	1	1	1	1	1
150	1.15	2	0.95	2.5	0.95	1.7	0.85	2.4	0.81	2.5	0.8	1.6

ΔV	Trinactin									
	0.01 M KCl		0.1 M KCl		0.5 M KCl		1 M KCl		4 molal KCl	
	G/G_{100}	I/I_{100}	G/G_{100}	I/I_{100}	G/G_{100}	I/I_{100}	G/G_{100}	I/I_{100}	G/G_{100}	I/I_{100}
25	1.15	0.18	1.16	0.18	1.2	0.15	1.28	0.17	1.4	0.18
50	1.1	0.36	1.1	0.29	1.13	0.33	1.18	0.34	1.27	0.4
100	1	1	1	1	1	1	1	1	1	1
150	0.9	2.1	0.89	2.9	0.87	2.3	0.84	2.2	0.81	2.1

ΔV	Trinactin											
	0.01 M NH_4Cl		0.02 M NH_4Cl		0.1 M NH_4Cl		1 M NH_4Cl		2 molal NH_4Cl		6 molal NH_4Cl	
	G/G_{100}	I/I_{100}	G/G_{100}	I/I_{100}	G/G_{100}	I/I_{100}	G/G_{100}	I/I_{100}	G/G_{100}	I/I_{100}	G/G_{100}	I/I_{100}
25	1.4	0.15	1.4	0.2	1.4	0.2	1.4	0.2	1.4	0.18	1.5	0.17
50	1.28	0.34	1.28	0.41	1.28	0.39	1.32	0.37	1.33	0.37	1.34	0.44
100	1	1	1	1	1	1	1	1	1	1	1	1
150	0.8	2.2^+	0.8	2.1	0.8	2.1	0.8	2.1	0.8	2.4	0.8	2.2

TABLE IV

THE POTENTIAL DEPENDENCE OF $k'_{is}+k''_{is}$ AS MEASURED BY CHANGES IN τ

Under each concentration the first column, I, contains experimental values of $\alpha/(\alpha+1)$ while the second, II, contains

$$\frac{k'_{is}(\Delta V)+k''_{is}(\Delta V)}{k'_{is}(100)+k''_{is}(100)}=\frac{\tau(100)\alpha(\Delta V)[\alpha(100)+1]}{\alpha(100)\tau(\Delta V)[\alpha(\Delta V)+1]}$$

ΔV	Nonactin-NH_4Cl				Trinactin-KCl			
	2 molal NH_4Cl		6 molal NH_4Cl		1 M KCl		4 molal KCl	
	I	II	I	II	I	II	I	II
25	0.68	0.48	0.86	0.45	0.71	0.46	0.89	0.49
50	0.73	0.58	0.89	0.55	0.74	0.56	0.91	0.58
100	0.83	1	0.93	1	0.85	1	0.94	1
150	0.92	1.9	0.95	1.77	0.92	2.05	0.97	1.8
200		Approx. 3.2						Approx. 3.3

ΔV	Trinactin-NH_4Cl							
	0.01 M NH_4Cl		0.1 M NH_4Cl		1 M NH_4Cl		6 molal NH_4Cl	
	I	II	I	II	I	II	I	II
25	0.8	0.43	0.8	0.45	0.91	0.54	0.97	0.52
50	0.84	0.51	0.82	0.6	0.91	0.59	0.98	0.59
100	0.92	1	0.89	1	0.95	1	0.99	1
150	0.95	1.9	0.93	1.9	0.97	1.9	1	2
200						Approx. 3.8		Approx. 3.3

Indeed such a determination in the present instance would require the detection of both relaxations. Thus if k_s were less than k_{is} there would be two relaxations at higher electrolyte activities and the slower time constant would reflect the value of k_s. However, since only one relaxation is observed the numerical calculations yield only that $k_s/k_{is} > 1$★. The insufficiency of the existing data is a result of the particular values of the rate constants. Unfortunately, the same difficulty arises in the interpretation of the data in each of the published studies on valinomycin. The ambiguity in the interpretation of the data obtained using negatively charged phospholipid membranes [11] has been discussed elsewhere [4]. A clearer example may be seen using the data for valinomycin, Rb^+, and dipalmitoleoyl lecithin membranes at 5 °C taken from Fig. 2 of [8]. Thus if it is assumed that k_{Ri}, k_{Di}, and k_s are independent of potential and that

$$k'_{is}+k''_{is} = 2k_{is}\cosh(0.5F\Delta V/RT), \tag{18}$$

★ If the data for nonactin–K^+ is interpreted literally according to the model, the concentration dependence of τ requires $k_s/k_{is} > 2$, while the curve fitting to the steady-state current voltage relation [17] suggests k_s/k_{is} is approx. 10–20. Any attempt to resolve the second relaxation will encounter formidable experimental difficulties since the time constant is expected to be approx. 1 μs or less and the amplitude less than approx. 0.05.

TABLE V

THE RATE CONSTANTS FOR CARRIER TRANSPORT BY NONACTIN AND TRINACTIN

			Nonactin			Trinactin		
			Na^+	K^+	NH_4^+	Na^+	K^+	NH_4^+
1.	k_{is}/k_{Di}		<0.02	0.04	0.25	0.02*	0.5	2
2.	$k_{Ri}k_{is}/k_{Di}k_s$	$[act]^{-1}$	0.01**	0.23	1.6	0.02**	2.5	10
3.	$\xi_s k_{Ri}k_{is}/k_{Di}+2k_{is}$	$[act]^{-1}\cdot cm\cdot s^{-1}$	$3.2\cdot 10^{-4}$	$3.2\cdot 10^{-2}$	$1.6\cdot 10^{-1}$	$2.1\cdot 10^{-3}$	$1.4\cdot 10^{-1}$	$2.3\cdot 10^{-1}$
4.	$\xi_s k_{Ri}k_{is}/k_{Di}$	$[act]^{-1}\cdot cm\cdot s^{-1}$	$3.2\cdot 10^{-4}$	$3.4\cdot 10^{-2}$	$2.3\cdot 10^{-1}$	$2.2\cdot 10^{-3}$	$2.8\cdot 10^{-1}$	1.16
5.	$\xi_s k_s$	$cm\cdot s^{-1}$	—	0.15	0.15	0.11**	0.11	0.12
6.	k_{Ri}/k_s	$[act]^{-1}$	>0.5**	5.8	6.4	1*,**	5	5
7.	k_{is}	s^{-1}	—	$2\cdot 10^4$	$7.5\cdot 10^3$	—	$2.3\cdot 10^4$	$1.5\cdot 10^4$
8.	k_{Di}	s^{-1}	—	$5\cdot 10^5$	$3\cdot 10^4$	—	$4.6\cdot 10^4$	$7.5\cdot 10^3$
9.	ξ_s		Approximate value $3\cdot 10^{-6}$ cm, see text					
10.	k_s	s^{-1}	$5\cdot 10^4$			$3.7\cdot 10^4$		
11.	k_{Ri}	$[act]^{-1}\cdot s^{-1}$	$2.5\cdot 10^4$**	$2.9\cdot 10^5$	$3.2\cdot 10^5$	$3.7\cdot 10^4$*,**	$1.85\cdot 10^5$	$1.85\cdot 10^5$

* A slight difference between the potential dependences of the rate constants for nonactin and trinactin could introduce a large error into the value of k_{is}/k_{Di} for trinactin–Na^+. However, the data of Ciani et al. [18] suggest that the value given is approximately correct.

** A slight variation with electrolyte activity of the potential dependence of the rate constants could introduce large errors into these values which are based on the curve fitting reported in the following paper.

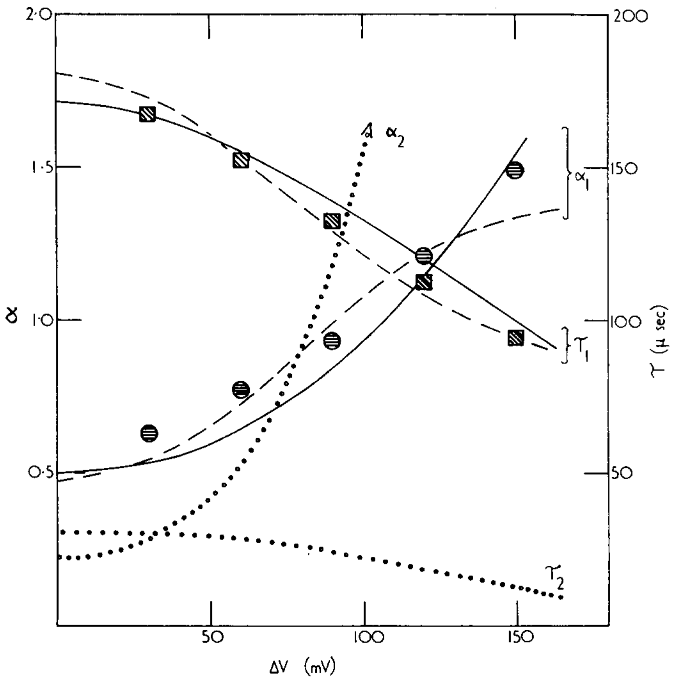

Fig. 6. A comparison of the experimental data for valinomycin, Rb^+, and dipalmitoleoyl lecithin + *n*-decane membranes at 5 °C (from [8]) with the relaxations predicted using Eqns A-13 to A-16, A-23, and A-24 with two differential trial assumptions for the potential dependence of $k'_{is}+k''_{is}$. The dashed and dotted curves are the slow and fast relaxations respectively predicted using $k_{is} = 2.9 \cdot 10^3\ s^{-1}$, $k_{is}/k_{Di} = 0.15$, $k_{Ri}k_{is}a_i/k_{Di}k_s = 0.4$, $k_s = 0.93\ k_{is}$, and $k'_{is}+k''_{is} = 2k_{is}\cosh(0.5\ F\Delta V/RT)$. The solid curves are predicted using $k_{is} = 10^3\ s^{-1}$, $k_{is}/k_{Di} \ll 1$, $k_{Ri}k_{is}a_i/k_{Di}k_s = 0.5$, $k_s = 100\ k_{is}$, and $k'_{is}+k''_{is} = 2k_{is}\cosh(0.3\ F\Delta V/RT)$. Another possible set of rate constants is given in the text.

then these data may be fitted accurately (see Fig. 6) using the slow relaxation predicted with $k_{is} = 2.9 \cdot 10^3\ s^{-1}$, $k_{is}/k_{Di} = 0.15$, $k_{is}k_{Ri}/k_s k_{Di} = 0.4\ M^{-1}$, and $k_s = 0.93\ k_{is}$. However, the theory then predicts a large, faster relaxation which has not been detected. If it is assumed instead, as is likely [15, 19] that $k'_{is}+k''_{is}$ increases less rapidly with potential, for instance as

$$k'_{is}+k''_{is} = 2k_{is}\cosh(0.3\ F\Delta V/RT) \qquad (19)$$

then the second predicted relaxation can be made neglibligy small and the data can be fitted using a variety of values for the constants, e.g. those in Fig. 6 or those indicated below. A similar reanalysis is possible for the data obtained using soya bean lipids [20].

Since it has not been possible to determine k_s, k_{Ri}/k_{Di} or ξ_s from the relaxation and flux measurements, it is necessary to seek alternative methods. Grell et al. [21] have titrated valinomycin adsorbed to dimyristoyl lecithin vesicles with KCl and RbCl at 25 °C and have reported that the apparent stability constants for both ions are 1 M^{-1}. If these values are accepted and it is assumed that they would be the same for valinomycin adsorbed to thin lipid membranes made from dipalmitoleoyl lecithin+n-decane, then the value of $k_{\mathrm{Ri}}/k_{\mathrm{Di}}$ for K^+ may be combined with the data given in Table III of the paper by Benz et al. [8] and the assumption in Eqn 19 for $k'_{\mathrm{is}}+k_{\mathrm{is}}''$ to give $k_{\mathrm{is}} = 1.2 \cdot 10^4\ \mathrm{s}^{-1}$, $k_{\mathrm{Di}} \gg k_{\mathrm{is}}$, $k_{\mathrm{is}}k_{\mathrm{Ri}}/k_{\mathrm{s}}k_{\mathrm{Di}} \simeq 0.5\ \mathrm{M}^{-1}$, $k_{\mathrm{s}} \simeq 2k_{\mathrm{is}}$, and a value $\beta_{\mathrm{s}} = 6.7 \cdot 10^{-3}$ cm for the aqueous phase to membrane adsorption constant for the carrier. Alternative assumptions for the potential dependence of $k'_{\mathrm{is}}+k''_{\mathrm{is}}$ will, of course, lead to different values for the constants (Stark, G., personal communication).

Unfortunately, no titration for nonactin or trinactin has been reported. The best technique presently available for estimating the adsorption is inaccurate and still open to objection. If nonactin or trinactin is added only to the aqueous phase, the conductance of a new membrane is very low but increases towards the steady-state value at a rate which depends markedly on the rate of stirring of the aqueous solution (apparent time constants range from approx. 30 s to several min) [7]. Thus, the flux of carrier into the membrane is at least partially limited by diffusion across the unstirred layer. For sufficiently low electrolyte activity that only a small proportion of the carrier is complexed, the initial rate of increase in carrier concentration follows

$$\frac{\mathrm{d}N_{\mathrm{s}}}{\mathrm{d}t} = \frac{D}{\delta}c_{\mathrm{a}} \tag{20}$$

where D/δ, the permeability of the unstirred layer to carrier, is estimated as $2 \cdot 10^{-4}$ cm/s [1, 7] and c_{a} is the aqueous concentration of carrier (in moles/cm^3). Still for negligible complex formation, the rate of change of the conductance of the membrane is, from Eqn 2

$$\mathrm{d}G/\mathrm{d}t = (z_{\mathrm{is}}^2 F^2/RT)[k_{\mathrm{Ri}}\,a_{\mathrm{i}}\,k_{\mathrm{is}}/(k_{\mathrm{Di}}+2k_{\mathrm{is}})](\mathrm{d}N_{\mathrm{s}}/\mathrm{d}t). \tag{21}$$

Thus

$$(\mathrm{d}G/\mathrm{d}t)_{t=0} \Big/ \left(\frac{z_{\mathrm{is}}^2 DF^2 c_{\mathrm{a}}\,a_i}{\delta RT}\right) = \frac{k_{\mathrm{Ri}}\,k_{\mathrm{is}}}{k_{\mathrm{Di}}+2k_{\mathrm{is}}}. \tag{22}$$

The rate of increase of conductance has been measured for 15 membranes with area of approx. 10^{-2} cm^2 using aqueous nonactin concentrations of 10^{-7}–10^{-8} M and 0.5 M NaCl with the result

$$k_{\mathrm{Ri}}\,k_{\mathrm{is}}/(k_{\mathrm{Di}}+2k_{\mathrm{is}}) = 30\text{–}100[\mathrm{act}]^{-1} \cdot \mathrm{s}^{-1}. \tag{23}$$

Similar experiments with trinactin and 0.1 M NaCl gave $1000 \pm 200\ [\mathrm{act}]^{-1} \cdot \mathrm{s}^{-1}$. Thus, since the steady-state conductances of nonactin and trinactin with NaCl are known (Table V), the estimates for the lipid solution–membrane adsorption constant become

$$\xi_{\mathrm{s}} = \begin{cases} 3 \cdot 10^{-6}\text{–}10 \cdot 10^{-6}\ \text{cm, nonactin} \\ 2.2 \cdot 10^{-6}\ \text{cm, trinactin.} \end{cases} \tag{24}$$

To the extent that the flux of the carrier into the membrane is partially limited by the interface, these are overestimates. An alternative procedure for estimating ξ_{s} and

$\xi_s k_{Ri}/k_{Di}$ is described in Appendix II. The uncertainty in ξ_s introduces an inversely related error into k_s and k_{Ri} since it is $\xi_s\, k_s$ and $k_{Ri}/2k_s$ which are known from the flux data.

Using the values of the constants listed in Table V, it is possible to calculate the concentration dependence of τ for each ion–carrier combination. For trinactin–NH_4^+ Eqn 11 is in agreement with the data. For trinactin–K^+, nonactin–NH_4^+, and nonactin–K^+ there is agreement between the data and numerical calculations. Those for nonactin–NH_4^+ display the nature of the predictions most clearly and are included in Appendix I.

At equilibrium a membrane of hydrocarbon or electrical thickness, d, contains $2\xi_s/d$ times as much carrier as the same volume of lipid solution. Thus $\xi_s = 3 \cdot 10^{-6}$ cm and $d = 3 \cdot 10^{-7}$ cm would imply that the carrier concentration in the membrane is 20 times greater. While this absorption of the carrier is not particularly strong, the concentration of complexes can be uncomfortably large. For $\xi_s k_{Ri}/k_{Di} = 7.5 \cdot 10^{-5}$ cm · act^{-1}, 1 M NH_4Cl and 10^{-3} M trinactin, the concentration on each side of the membrane would be $N_{is} = 4.5 \cdot 10^{-11}$ moles/cm^2 which corresponds to an area per molecule of 370 Å^2.

In this paper experimental data for nonactin and trinactin have been compared with a simple version of the carrier model. First attention was given to two aspects of the model which could be tested without knowledge of the number of carrier molecules in the membrane or of the potential dependence of the rate constants. Then an estimate of the carrier adsorption was obtained and this additional information allowed the specification of a complete set of rate constants and a third test of the model. In the following paper the dependence of the fluxes on ion and carrier concentrations and on the applied potential will be considered in greater detail.

APPENDIX I

The mathematical description of the model may be written down from the assumptions listed in the main text and the equations for the conservation of mass (see Fig. 1) [12, 22]:

$$\frac{dN'_{is}}{dt} = -k'_{is}\,N'_{is} + k''_{is}\,N''_{is} + k'_{Ri}\,a'_i\,N'_s - k'_{Di}\,N'_{is} \tag{A-1}$$

$$\frac{dN'_{s}}{dt} = -k'_{s}\,N'_{s} + k''_{s}N''_{s} - k'_{Ri}\,a'_i\,N'_s + k'_{Di}\,N'_{is} \tag{A-2}$$

$$\frac{dN''_{is}}{dt} = k'_{is}\,N'_{is} - k''_{is}\,N''_{is} + k''_{Ri}\,a''_i N''_s - k''_{Di}\,N''_{is} \tag{A-3}$$

$$\frac{dN''_{s}}{dt} = k'_{s}\,N'_{s} - k''_{s}N''_{s} - k''_{Ri}\,a''_i N''_s + k''_{Di}N''_{is}. \tag{A-4}$$

In the relaxation experiments only three of the surface concentrations are independent functions of time since

$$N'_s + N''_s + N'_{is} + N''_{is} = N_T \tag{A-5}$$

and N_T the total concentration of carrier in the membrane changes so slowly that it may be assumed constant.

First approximation

For $k'_{Ri}a'_i = k''_{Ri}a''_i \qquad$ (A-6)

and $k'_{Di} = k''_{Di} \qquad$ (A-7)

the sum of Eqns A-2 and A-4 may be written

$$d(N'_s + N''_s)/dt = k'_{Di}N_T - (k'_{Ri}a'_i + k'_{Di})(N'_s + N''_s) \tag{A-8}$$

which has a simple solution. However since k'_{Ri} and k''_{Ri} are likely to vary in opposite directions if they vary at all, the solution is most useful when trivial, i.e.

$$N'_s + N''_s = N_T/(1 + k_{Ri}a_i/k_{Di}) = 2N^0_s \tag{A-9}$$

which is a constant. Using Eqn A-9 and its parallel for $N'_{is} + N''_{is}$ to eliminate N''_s and N''_{is} and assuming $k'_s = k''_s$ there are two independent equations,

$$dN'_{is}/dt = 2k''_{is}(k_{Ri}a_i/k_{Di})N^0_s + k_{Ri}a_i N'_s - (k_{Di} + k'_{is} + k''_{is})N'_{is} \tag{A-10}$$

and

$$dN'_s/dt = 2k_s N^0_s + k_{Di} N'_{is} - (k_{Ri}a_i + 2k_s)N'_s, \tag{A-11}$$

which may be solved using standard methods. The general solution for each concentration and flux is of the form, written here for N'_s,

$$N'_s = {}^0N'_s + {}^1N'_s e^{-t/\tau_1} + {}^2N'_s e^{-t/\tau_2} \tag{A-12}$$

where

$$\lambda_1 = 1/\tau_1 = a - b, \qquad \text{(A-13)*}$$

$$\lambda_2 = 1/\tau_2 = a + b, \qquad \text{(A-14)*}$$

$$2a = 2k_s + k_{Ri}a_i + k'_{is} + k''_{is} + k_{Di} \tag{A-15}$$

$$2b = \{(k'_{is} + k''_{is} + k_{Di} - 2k_s - k_{Ri}a_i)^2 + 4k_{Ri}a_i k_{Di}\}^{\frac{1}{2}} \qquad \text{(A-16)*}$$

It follows immediately from (A-16) that

$$2b > |k'_{is} + k''_{is} + k_{Di} - 2k_s - k_{Ri}a_i| \qquad \text{(A-17)*}$$

and thus

$$\left.\begin{aligned} \lambda_2 &> k_{Ri}a_i + 2k_s, \\ \lambda_2 &> k'_{is} + k''_{is} + k_{Di}, \\ \lambda_1 &< k_{Ri}a_i + 2k_s, \end{aligned}\right. \tag{A-18}$$

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[*] The b in Eqns A-13 to A-17 is consistent with the notation of Stark et al. [6, 11]. It is not the same as the b in the exponential of Eqn 17.

and

$$\lambda_1 < k'_{is}+k''_{is}+k_{Di}.$$

The coefficients in Eqn A-12 for each concentration and flux may be calculated from the values of the unknowns at one specific time. Just after the sudden application of a potential, the concentrations have not had time to change and thus

$$ {}^0N'_s+{}^1N'_s+{}^2N'_s = N^0_s \tag{A-19}$$

and

$$ {}^0N'_{is}+{}^1N'_{is}+{}^2N'_{is} = (k_{Ri}\,a_i/k_{Di})N^0_s. \tag{A-20}$$

The algebra which remains to be done after the expressions such as Eqn A-12 are substituted into Eqns A-10 and A-11 is considerably simplified if ${}^0N'_s$ and ${}^0N'_{is}$ are expressed in terms of J_∞,

$$ {}^0N'_s = N^0_s-J_\infty/2k_s \tag{A-21}$$

$$ {}^0N'_{is} = (k_{Ri}\,a_i/k_{Di})N^0_s-[(2k_s+k_{Ri}\,a_i)/2k_s k_{Di}]J_\infty. \tag{A-22}$$

After some manipulation the results are

$$\alpha_1 = \frac{{}^1J_{is}}{J_{is}(\infty)} = \frac{k'_{is}+k''_{is}}{2k_s k_{Di}}\left\{\frac{\lambda_2(k_{Ri}\,a_i+2k_s-\lambda_1)}{\lambda_2-\lambda_1}\right\} \tag{A-23}$$

and,

$$\alpha_2 = \frac{{}^2J_{is}}{J_{is}(\infty)} = \frac{k'_{is}+k''_{is}}{2k_s k_{Di}}\left\{\frac{\lambda_1(\lambda_2-k_{Ri}\,a_i-2k_s)}{\lambda_2-\lambda_1}\right\} \tag{A-24}$$

If either of the λ's approaches $k_{Ri}a_i+2k_s$ the corresponding α approaches zero and the other λ approaches $k'_{is}+k''_{is}+k_{Di}$. The conversion of Eqns A-23 and A-24 to the forms given by Stark et al. [11] is straightforward particularly after it is noticed that

$$-\lambda_1\lambda_2 = k_{Ri}\,a_i\,k_{Di}-(k_{Ri}\,a_i+2k_s)(k'_{is}+k''_{is}+k_{Di}). \tag{A-25}$$

Benz et al. [8] have pointed out that the assumption that the carrier is adsorbed to the surfaces of the membrane may be relaxed by introducing a notional concentration of carrier in the centre of the membrane. Provided that k_{Ri}, k_{Di}, and k_s are independent of potential, their result follows naturally from the derivation given above. Thus $N'_{is}+N''_{is}$ and $N'_s+N_s^c+N''_s$ are constants, and via the same device N_s^c is constant. Eqn A-10 is unchanged and Eqn A-11 may be recovered but with $2k_s$ changed to k_s^i where k_s^i is the rate constant for carrier jumping into the centre. The solution must therefore be identical except for this substitution and a minor change in Eqn A-5

$$N_T-N^c_s = N'_s+N''_s+N'_{is}+N''_{is} \tag{A-26}$$

The approximate expressions in the text Eqns 10–15 correspond to the series expansions of the expression in Eqn A-16 whenever the squared term is much

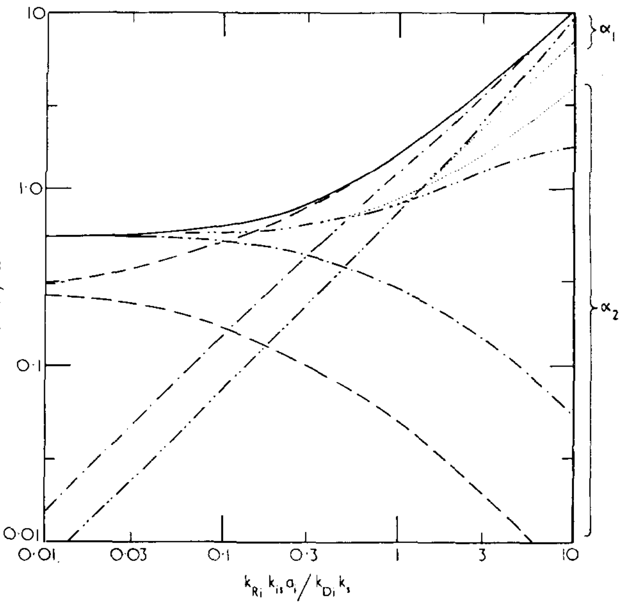

Fig. 7. The theoretically predicted dependence (Eqns A-23 to A-24) of the relaxation amplitudes at 25 mV on the electrolyte activity for $k_{is}/k_{Di} = 0.25$ and various values of k_s/k_{is}: 10 (—), 3 (– – –), 1 (– · –), 0.1 (– · · · –), and 0.001 (· · ·). For $k_s/k_{is} = 10$, α_2 is always less than 0.01.

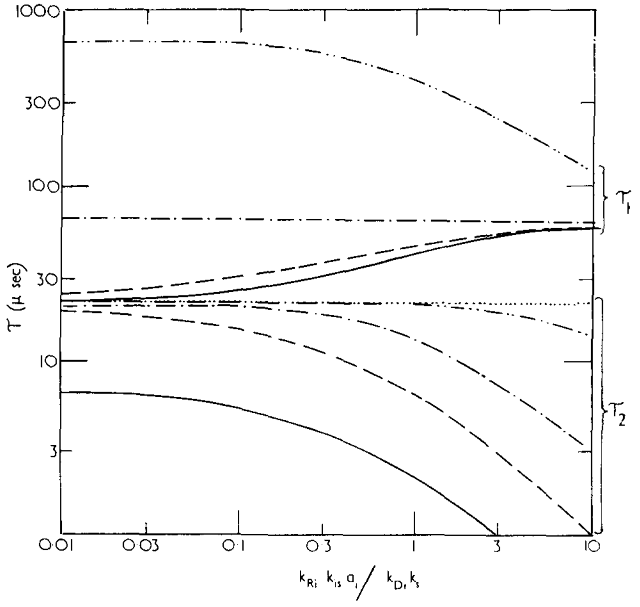

Fig. 8. The theoretically predicted dependence (Eqns A-13 and A-14) of the time constants on the electrolyte activity for $k_{is}/k_{Di} = 0.25$, $k_{is} = 7.5 \cdot 10^3\ s^{-1}$ and various values of k_s/k_{is}: 10 (—), 3 (- -), 1 (- · -), 0.1 (- · · · -), and 0.001 (· · ·). The value of τ_1 at low activity for $k_s/k_{is} = 0.001$ is $6.6 \cdot 10^4$ μs. Both τ's are inversely proportional to k_{is}.

larger than $4k_{Ri}a_ik_{Di}$. Those approximations are useful for extreme values of k_s/k_{is}, but comparison with the experimental data requires that the α's and τ's be known for intermediate values as well. Eqns A-13 to A-16, A-23 and A-24 are easily solved for test values and a sample of such solutions for $k_{is}/k_{Di} = 0.25$, $k_{is} = 7.5 \cdot 10^3\,s^{-1}$, and $k'_{is}+k''_{is}$ as indicated in Eqn 17 are displayed in Figs 7 and 8. If k_{is}, k_{is}/k_{Di}, $k_{is}k_{Ri}/k_sk_{Di}$, and k_s/k_{is} (or k_{Ri}/k_{Di}) are chosen as the independent parameters, then k_{is} merely changes all values of the time constants proportionally and has no effect on the amplitudes.

In using Figs 7 and 8 it is important to remember that very accurate data are required to resolve relaxations for which the time constants differ by much less than an order of magnitude. Thus, as noted by Stark et al. [11], under some circumstances there may be two relaxations present even though the data can be fitted as a single exponential. For example in fitting the data for nonactin–NH_4^+, if $k_s/k_{is} = 0.1$, as $k_{Ri}a_i$ is increased a second slower relaxation should become obvious, if $k_s/k_{is} = 10$ only one relaxation is predicted and this one behaves as required, while if $k_s/k_{is} = 1$ there are two relaxations which cannot be resolved and the apparent relaxation acts as if k_s/k_{is} were greater than 10.

Second and third approximations

For very low electrolyte activities such that

$$k_{Ri}\,a_i(k'_{is}+k''_{is})/k_{Di} \ll 2k_s \tag{A-27}$$

and

$$k_{Ri}\,a_i/k_{Di} \ll 1 \tag{A-28}$$

the concentration of unbound carrier on each side remains constant. Thus in the interesting limit where $k'_{is} \gg k''_{is}$, the solution is simply

$$I(t) = z_{is}FN_{is}\frac{k'_{Ri}\,a'_i\,k'_{is}}{k'_{is}+k'_{Di}} + \left[\frac{k'_{Ri}\,a'_i\,k'_{is}}{k_{Di}} - \frac{k'_{Ri}\,a'_i\,k'_{is}}{k'_{is}+k'_{Di}}\right] e^{-t(k'_{is}+k'_{Di})} \tag{A-29}$$

If k'_{Ri}/k'_{Di} can increase with potential, the steady-state current may even exceed the initial current. It is difficult to obtain data to test Eqn A-29 because the currents are small.

For electrolyte activities sufficiently high that

$$k_{Ri}\,a_i(k'_{is}+k''_{is})/k_{Di} \gg 2k_s \tag{A-30}$$

and

$$k_{Ri}\,a_i/k_{Di} \gg 1 \tag{A-31}$$

all but the end of the relaxation is solely the rearrangement of preformed complexes between the two sides of the membranes. Thus the time course is approximately

$$I(t)-I_\infty = z_{is}F(N_T/2)(k'_{is}-k''_{is})\,e^{-t(k'_{is}+k''_{is})} \tag{A-32}$$

The limiting form for $1/\tau$ from Eqn 11 is also $k'_{is}+k''_{is}$.

APPENDIX II

A determination of ξ_s and $\xi_s k_{Ri}/k_{Di}$ completely independent of flux measurements is possible in principle by the use of surface tension measurements to calculate the adsorptions (cf. [16]). Unfortunately the change in the tension of the interface between 1 M NaCl and glyceryl monooleate+n-hexadecane caused by adding 10^{-3} M trinactin to the lipid phase is below the level of reproducible detection, approx. 0.2 dyne/cm^2, corresponding to $N_s < 6 \cdot 10^{12}$ molecules/cm^2 (Haydon, D. A. and Hladky, S. B., unpublished). Changes in concentration of the surface active material can, however, be detected by changes in the surface or compensation potential using the procedures described in [23]. Spreading glyceryl monooleate+n-hexadecane on 0.1 M NaCl gives a compensation potential, ΔV_{gmo}, of 321 mV while addition of 10^{-3} M trinactin to the lipid increases the potential by 10 mV. Spreading the lipid solution on MNH_4Cl results in 326 mV with an increase of 27 mV if 10^{-4} M trinactin is added to the lipid and 107 mV for 10^{-3} M trinactin (Haydon, D. A. and Hladky, S. B., unpublished). The less than proportional increase in potential as trinactin concentration is increased could be explained by interaction between the complexes.

In the simplest physical model (see [24]) the compensation potential is produced by the summation of the dipole moments of the various groups at the interface*. Estimates of the increments in potential can be calculated from the observed potentials for the various components and the areas per molecule. Thus for glyceryl monooleate ($A_0 = 40$ Å^2 [25]) the increment per molecule within unit area would be $\Delta V_{gmo} = 321 \times 40 \times 10^{-16} = 1.3 \cdot 10^{-12}$ mV · cm^2/molecule. Similar values for uncomplexed and complexed trinactin can be based on the results of Kemp and Wenner [26] who found at the air–water interface that $A_{tri} \simeq A_{tri-NH_4} \simeq 125$ Å^2 with compensation potentials of 600 mV for trinactin and 820 mV for trinactin–NH_4^+ complexes. The increments are $\Delta V_{tri} = 7.5 \cdot 10^{-12}$ mV · cm^2/molecule and $\Delta V_{tri-NH_4^+} = 1.0 \cdot 10^{-11}$ mV · cm^2/molecule, respectively. It is assumed that in order for trinactin to adsorb an equal area of lipid must be displaced. Therefore the increments for increase in potential per molecule added become $\Delta V_{tri} = 3.4 \cdot 10^{-12}$ mV · cm^2/molecule and $\Delta V_{tri-NH_4^+} = 5.9 \cdot 10^{-12}$ mV · cm^2/molecule. Using these values N (NaCl) $= N_s = 3 \cdot 10^{12}$ molecules/cm^2 at 10^{-3} M trinactin (presumably $N_s = 3 \cdot 10^{11}$ molecules/cm^2 at 10^{-4} M) and $N(NH_4Cl)-N_s = (k_{Ri}a_i/k_{Di})N_s = 1.6 \cdot 10^{13}$ molecules/cm^2 at 10^{-3} M and $4.4 \cdot 10^{12}$ molecules/cm^2 at 10^{-4} M. These calculations therefore lead to $\xi_s = 5 \cdot 10^{-6}$ cm and $\xi_s k_{Ri}/k_{Di} = 7.3 \cdot 10^{-5}$ cm · [act]$^{-1}$. The latter value is in good agreement with that calculated from the flux data (Rows 4 and 7 in Table V) while the former is in agreement with the value found from the initial rate of increase of conductance (Row 9). Nevertheless there is little reason to accept the assumptions in this calculation.

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* For the present purpose the dipole moment for the trinactin–NH_4^+ complex is taken to include a contribution due to the separation of the complex from the Cl^- counterion.

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